Comm.
Dr. Gardner
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CHRONIC PULMONARY DISEASES #901

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 50TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application for Research Grant (Use extra pages as needed)

JAN 3 1 1973

Date: 1/15/73

1. Co-Principal Investigators (give title and degrees):
GEORGE WEINBAUM, PH.D, Bioscientist, Pulmonary Disease Section
PHILIP KIMBEL, M.D., Head, Pulmonary Disease Section

2. Institution & address:
Albert Einstein Medical Center
Northern Division
York & Tabor Roads
Philadelphia, Pa. 19141

3. Department(s) where research will be done or collaboration provided:
Pulmonary Disease Section and
Research Laboratories
Albert Einstein Medical Center

4. Short title of study: THE EFFECT OF AIR-BOURNE POLLUTANTS ON LUNG PROTEINASE: ANTIPROTEINASE LEVELS

5. Proposed starting date: July 1, 1973.

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6. Estimated time to complete: 3 years.

7. Brief description of specific research aims:

The twin objectives of this investigation are 1) to identify and quantitate possible factors responsible for protecting the lung against the action of autogenous proteinases - previously shown in this laboratory to produce experimental emphysema in dogs - and 2) to examine the role of cigarette smoke and other pollutants on this interaction. We shall isolate, quantitate and characterize the normally occurring substances, found in lung tissue and sera of several species of animals, which inhibit the activity of proteolytic enzymes. These data will then be compared both with the incidence of spontaneous emphysema and with the ease of experimental emphysema induction in these species. The production of antiproteinases and their ability to interact with enzymes able to induce experimental emphysema in animals will be studied and evaluated in normal animals and those exposed to cigarette smoke or nitrogen dioxide. Both of these components are known air pollutants and the latter agent has been shown to cause emphysematous lesions in animal models.

Using experimental pollutant and nonpollutant conditions we will look for qualitative and quantitative changes in levels or activities of autogenous proteinases and anti-proteinases and determine the relative effectiveness of emphysema induction in the dog model system using leukocyte or macrophage proteinases in both conditions.

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8. BRIEF STATEMENT OF WORKING HYPOTHESIS:

Our working hypothesis is that emphysema may be induced by the proteolytic activity of specific enzymes from polymorphonuclear leukocytes or pulmonary macrophages. The release of these enzymes may be stimulated by various air-borne pollutants. These enzymes overwhelm the local defense mechanisms in the lung, including such factors as serum and tissue antiproteinases, and destroy or alter the elastic tissue of the alveoli.

9. DETAILS OF EXPERIMENTAL DESIGN AND PROCEDURES

PART I - INTRODUCTORY STATEMENT

Epidemiologic studies suggest that smoking is an important factor in the development of pulmonary emphysema in humans. Two major, non-mutually exclusive theories for its induction differ only in their emphases on initial tissue attack. In the vascular theory (1), the initiating lesion is of vascular origin; resulting in obstruction of branches of the bronchial or pulmonary blood supply. The subsequent deficiency of nutrients would then lead to necrosis of alveolar walls and septa to form the characteristic emphysema pathology. According to the second theory (2), emphysema develops due to the direct attack of proteolytic enzymes at the air-lung interface. Regardless of which theory is correct there is eventual extensive damage to both the vascular and parenchymal tissue. A logical source of this degradation could conceivably be intracellular collagenase and/or elastase, released into the surrounding tissues due to cellular necrosis caused by such stimuli as chronic infection or chronic pollution-induced destruction.

Although cigarette smoking and human emphysema have been statistically related using clinical and autopsy material, it has not yet been shown if there is a direct causal relationship or if smoking and other pollutants merely accelerate a normal aging process. Also, if there is a direct relationship between them it is still possible that smoking is but one of a number of extrinsic factors able to act synergistically with autogenous agents to effect this lung damage. Those experimental studies which have been performed on the effects of smoking do, however, suggest reasons for exploring the action of cigarette smoke as a single contributing factor in emphysema induction.

The response of the lungs to nitrogen dioxide, especially to chronic low levels, should similarly be investigated using the same criteria used for smoking. Not only has this very simple compound been shown to produce emphysema (3), its occurrence in cigarette smoke and other complex forms of pollution suggest its use as a model causative compound of simple structure.

When human pulmonary leukocytes from smokers and non-smokers were compared (4, 5, 6), even asymptomatic smokers had greatly increased numbers of primarily polymorphonuclears and macrophages with a direct cor-

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relation to the amount of smoking and smoke inhaled. That this is not simply a protective effect, i.e., more phagocytes are supplied to remove an increased amount of foreign matter, was demonstrated by the finding that there was an actual decrease in the antibacterial activity of alveolar macrophages from smokers. This effect differed among the various brands tested and varied inversely with the effectiveness of filtration (7). The significance of this particular change in capability, that is, the decrease in antibacterial activity as opposed to such other findings as change in size or pigmentation, lies in the ability of the phagocytosed particles to cause the lysis of the engulfing leukocyte with subsequent release of enzymes able to degrade pulmonary tissue.

The demonstrated greater susceptibility of cigarette smokers to respiratory infections may, therefore, be due to this combination of insufficient leukocyte degradation of invading organisms and the damage resulting from the liberated proteases on lung tissue (8). Since studies of human pulmonary emphysema are hampered by time required for induction and moral considerations, animal models have been employed. Although the horse has the advantage of having lung anatomy, distribution of bronchial arteries, and a natural emphysema similar to that found in humans, economy dictated that another animal model be used. Since this laboratory has successfully produced emphysema-like lesions in dogs (9), using aerosolized leukocyte proteinases and since the parenchymal effects of smoking in dogs resembled human emphysema (10) this system will be used.

It has been suggested by many groups (11) that a decreased level of serum alpha$_1$ antitrypsin is of primary importance in correlating with the development of hereditary emphysema. Other investigators (8,12), however, have been unable to demonstrate a relationship between the development of emphysema and smoking in those having intermediate or normal levels of this antiprotease. Although this may be a characteristic of the species studied, the report (13) that lung tissue binds the serum antiprotease suggests that not only is the total level of antiprotease important but also its localization and its availability to bind and thus inhibit proteolytic enzymes. Material from fractionated lung tissue must be examined for its role in proteinase-antiproteinase balance.

With these data in mind, we propose the following research program.

PART II

A. RESEARCH PLAN OUTLINE

1. To Characterize Antiproteinase(s) in the Lung.

a. Fractionate lung tissue from several mammalian species and isolate and characterize the factor(s) responsible for antiproteinase activity.

b. Comparison of the factor(s) found in the lung with serum proteins demonstrating antiproteinase activity.

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2. To Determine the Role of Smoking and Other Pollutants on the Interaction between the Proteinases and Antiproteinases in the Lung.

a. In vitro assay of proteinases from alveolar macrophages, lung tissue; and PMN's and the qualitative and quantitative effects on them due to exposure to cigarette smoke or nitrogen dioxide.

b. In vitro assay of serum and lung antiproteinases from normal and pollutant-treated dogs.

c. Comparison of ease of induction of experimental emphysema using material from normal and pollutant-treated dogs on both in vivo and in vitro levels.

B. EXPERIMENTAL PROCEDURE

1. Characterization of Lung Antiproteinases

a. Species variations and relationship to spontaneous emphysema.

Lung tissue from normal guinea pigs, hamsters, humans, horses and dogs will be examined for substance(s) able to inhibit any of the proteolytic enzymes found in polymorphonuclear leukocytes. These particular species were chosen for the following reasons. In a study by Ihrig, et al (14), guinea pigs had the highest and hamsters the lowest levels of serum antitryptic activity of those animals tested. Since preliminary results in this laboratory (15) indicate differences in the ability of these animals to develop emphysema following exposure to papain we believe a better characterization of the various lung protease inhibitors is warranted. Human material will be studied not only as this is the species of primary concern, but also because no clear-cut relationship has as yet been found between the intermediate levels of serum proteinase inhibitor and development of emphysema in smokers (12). One approach, that of genetic analysis of the numerous alleles involved in production of serum alpha$_1$ antitrypsin is being undertaken in several other laboratories (13). Our approach is a biochemical one in which we will evaluate the role of proteinase:antiproteinase balance under pollutant and non-pollutant conditions in relation to the development of experimental emphysema. Horse tissue will be examined as this species is one of the only two (along with the rabbit), other than man, recorded as developing spontaneous emphysema. Additional considerations suggesting that the horse is a useful model are (a) its level of serum inhibitor is approximately the same as the level in humans; (b) lung anatomy in the horse more nearly resembles that in man; (c) the time course of emphysema development, adjusted to human years, is quite similar between the two species. Dog material will be employed due to the considerable body of data already available from this lab using this animal. Also, a direct relationship was found between macroscopic parenchymal disruption in dogs and duration of daily cigarette smoking over a period of 0-22 months (16). Microscopically, these lesions resembled human emphysema (16). Emphysema has been successfully produced in our laboratory in vivo

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using papain or leukocyte homogenate instillation or aerosolization. We have also developed an *in vitro* system for emphysema production utilizing isolated lobes and instillation of proteinases.

Isolation and Quantitation of Tissue Inhibitors

Lung tissue from the various species will be minced, homogenized in a blender, and washed three times with water followed by centrifugation at 15000 rpm (15). The pellet will be washed twice, including once overnight, with 1 M NaCl. The sodium chloride supernate will then be recentrifuged at 42,000 rpm (15). Supernate extracts will be assayed for their ability to inhibit the proteolytic activity of enzymes extracted from polymorphonuclear leukocytes using the hemoglobin assay (17). It is expected that antiproteinase activity will be found in this fraction because of the preliminary work of Lieberman (18) and Janoff (19). We will initiate this work using the NaCl extract, but will check other lung extraction procedures also.

Characterization of Inhibitors

In order to characterize these antiproteinases they will be purified using such standard procedures as precipitation with ammonium sulfate, methanol, trichloracetic or perchloric acid and various types of ion-exchange chromatography (20). An alternative procedure will be sought using the principle of affinity chromatography (21). Crystallin trypsin will be bound to Sepharose 4B using the cyanogen bromide method of Cuatrecasas and Anfinsen (22), and the matrix poured into a column. Lung extract containing the antiproteinase will then be applied to the column, nonbound protein washed off, and the adhering inhibitor selectively eluted.

Effect of Inhibitors on Induction of In Vitro Emphysema

In vitro emphysema has been induced in our laboratory using isolated lobes of dog lungs. Following removal of the lungs from the body the individual lobes were instilled with solutions of prdeinase(s) and the enzyme(s) allowed to digest the tissues for 1½ hours at room temperature. We will determine if the inhibitors isolated above will affect *in vitro* induction. Formaldehyde will be instilled into the lungs at a standard pressure of 25 cm. The organs will be immersed in formaldehyde for 48 hours, mounted, sliced and stained. Sections will be examined for alveolar wall destruction using the method of Dunhill (23). This procedure will allow us to better define what we are considering to be emphysema-like lesions and also to quantitate the effects of various doses of agents required to produce a defined level of lung destruction. The use of isolated lobes will provide us with a considerable economic advantage over *in vivo* work employing the entire animal. It will also allow us to minimize the effect of any animal to animal variation on a given experiment.

b. The Relationship between lung and serum proteolytic inhibitors

Although both the serum and the pulmonary tissues have been reported to contain substances able to inhibit proteolytic enzymes no one has studied the possible relationship between the inhibitors from

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these two sources. The value of such a comparison lies in the relationship of these inhibitors to both the etiology and prognosis of the disease state, always with the assumption that since proteolytic enzymes are significant in these processes so too are these inhibitors. If a serum inhibitor is actually a subunit of a lung inhibitor (or vice versa) then it could be expected that there would be some sort of quantitative relationship between their concentrations. Conditions regulating this ratio could be involved in pathogenesis of pulmonary emphysema. Genetic abnormalities affecting the specific regulatory gene(s) would also be expected to affect both serum and pulmonary inhibitor if they were derived from the same molecule. Conversely, if the lung and serum antiproteinases are not subunits of some primal molecule any relationship between the concentration of one vs. the other could have some non-genetic, e.g., environmental origin.

Several methods will be employed to study the relationship between antiproteinases of the lung and the serum. Lung antiproteinases isolated as described in specific aim"a"will be examined using polyacrylamide gel electrophosesis, with and without sodium dodecyl sulfate, to determine if these agents isolated from different species have subunits such as easily dissociable polypeptide chains and if they are similar to serum antiproteinases isolated in a similar chromatography procedure. Antisera will be raised in rabbits injected with purified serum antiproteinase and tested against lung antiproteinase using the immunoelectrophoretic and Ouchterlony techniques to find out whether or not the molecules share antigenic, and therefore, structural sites. Samples of each inhibitor will also be hydrolyzed and subjected to two-dimensional electrophoresis and chromatography, producing two-dimensional peptide maps in order to further compare the primary structure of the proteins. Each inhibitor will be tested against such standard proteolytic enzymes as trypsin, collagenase, papain and elastase using hemoglobin and other pure proteins as substrates to obtain a general idea of its inhibitory spectrum. Each inhibitor will also be tested for its effect on enzymes derived from a given species' polymorphonuclear leukocytes and macrophages, using as substrates material obtained from fractionated lung tissue to more closely approximate its actual effect in the intact animal.

2. The Role of Cigarette Smoke and Nitrogen Dioxide in Determining Proteinase:Antiproteinase Interaction

a. Effect of Smoking or Nitrogen Dioxide on Proteinases from Lung Macrophages, Lung Tissue, and Polymorphonuclear Leukocytes.

The response of lung tissue to both acute and chronic treatments with cigarette smoke and nitrogen dioxide, have been amply described micro- and macroscopically. Several laboratories (10,16) have supplied data detailing the numerous changes resulting from in vivo exposure of pulmonary tissue to these agents but none has sought to determine their effects on the interaction between autogenous proteinases and antiproteinases.

Populations of lung macrophages will be obtained by lavage (4). PMNs will be obtained from whole blood by the usual procedures used in our

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laboratory (24). Lung cells will be prepared by mincing lung tissue and homogenizing in a Potter-Elvehjem homogenizer, disrupted by sonication, and made into acetone powders. The powder will be extracted using water, 1 M NaCl, and 8 M urea to effect an initial fractionation corresponding roughly to acid, neutral, and alkaline proteinases. Work currently in progress in our laboratory using the PMN material from dog blood indicates: 1) the water extract is much richer in acidic proteinase (catheptic) activity than the combined neutral and alkaline activities; 2) the acidic activity is much less in the sodium chloride extract and essentially zero in the urea extract; 3) the sodium chloride extract has the greatest amount of alkaline and neutral activities; 4) the neutral and slightly acidic (pH 5) activities of the urea extract are more significant than either the acidic or alkaline proteolytic activities; 5) elastolytic activity, using Boc-L-alanine ester as substrate, is significant only in the fraction of the urea extract subsequently precipitated between 20 and 40% saturated ammonium sulfate; 6) collagenase activity, using bovine achilles tendon as substrate was found in both the water and the sodium chloride extract, with minimal activity in the urea extract. These findings are important in that they 1) demonstrate an initial significant separation of the major proteolytic activities and 2) the two principal enzymatic activities involved in connective tissue destruction, i.e., elastase and collagenase, and implicated in causing disease in these tissues (25) are easily separated. Although routine elastase assays are performed using a synthetic substrate, the use of elastin-orcein has confirmed the fact that the urea fraction precipitated by 20-40% ammonium sulfate did indeed have elastolytic activity.

In order to define which of these activities are important in emphysema induction we are preparing various fractions of lung tissue for use as more specific substrates for these enzymes. Those enzymes capable of destroying tissue-derived substrates will be employed in those studies dealing with proteinase-antiproteinase interactions.

Macrophages will be washed from lungs of dogs exposed to cigarette smoke or nitrogen dioxide. The pollutant exposure will be performed using sealed chambers into which the dog is placed and the appropriate pollutant piped in. We will also use intubated, anesthetized dogs in which the pollutant will be directly instilled into the lungs. Acute treatment will consist of 1-6 hours exposure to cigarette smoke adjusted to give an amount equivalent to two packs for an average weight smoker; or exposure to nitrogen dioxide at 30 ppm. Chronic treatment using cigarette smoke adjusted to give an amount equivalent to one-half pack in one group and two packs in a second group; or exposure to nitrogen dioxide at 2 ppm for one group and 17 ppm for another group (26) for varying intervals of time over a 0-6 month period.

The proteolytic activity of the macrophages will be determined using standard protein and synthetic substrates and also the specific lung substrates described above. Proteolytic activity will be measured not only using acetone powders of sonicated cells, but also from supernatants of cells allowed to merely discharge their enzymes due to possible changes in intracellular stability. These determinations of proteolytic activity will be performed on cells from both normal and treated dogs since there are reports (27) that both smoking and nitrogen dioxide treatment cause an increase in pulmonary proteolytic activity. Such data should be important in ascertaining if certain pollutants can potentiate the development of emphysema via the mechanism of proteolytic degradation.

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b. The Effect of Smoking or Nitrogen Dioxide on Serum and Lung Anti-proteinase Levels and Activities.

Sera from dogs subjected to acute and chronic smoking or nitrogen dioxide schedules will be assayed for changes of antiproteolytic activities using both the antitryptic assay of (28) and the more specific anzyme-substrate systems described above. Inhibition of specific anti-proteolytic activities will be followed using the agar gel electrophoretic technique of Ouchterlony (29), in order to demonstrate not only different levels, but also differemt inhibiting spectra of antiproteolytic agents. These data will, therefore, aid in determining if cigarette smoke or nitrogem dioxide affect the level or composition of the various serum anti-proteinases.

Lung antiproteinase isolated as described in an earlier section will be assayed to determine possible changes due to exposure to either cigarette smoke or nitrogen dioxide. The total amount of lung antiproteinase, the amount bound in the lung to proteinases and the ratio of these values will be determined to give a quantitative picture of the relationship of pollutants to antiproteinase activity. Qualitative differences will be sought for by redetermining the spectra of enzymes inhibited by lung antiprotein-ase after exposure to cigarette smoke or nitrogen dioxide and comparing these to normal values.

c. Effect of Cigarette Smoke or Nitrogen Dioxide on Induction of Proteinase-Induced Emphysema.

Emphysema induction will be studied both in vivo and in vitro. Initially, our studies using PMN homogenates to produce emphysema will be extended to dogs previously subjected to various regimens of smoking or exposure to nitrogen dioxide as described in the preceding section. In our acute in vivo studies dogs exposed to cigarette smoke or nitrogen dioxide will be treated with various amounts and types of leukocyte-derived proteinase, sacrificed, and the severity of emphysematous lesions compared to those of untreated dogs. Chronic studies, as described previously, will also be performed prior to proteinase treatment to measure the short and long term effects of exposure to these agents on the ease of emphysema-induction using leukocyte proteinases.

In vitro studies will be carried out by removing lungs from dogs exposed to cigarette smoke or nitrogen dioxide and the isolated lobes used as test organs for proteinase studies. This method is not only more economical than whole animal studies but also emphasizes the effects of pollutants and proteinases on the lungs themselves, with a minimum of extra-pulmonary involvement due to circulatory transport of serum factors to act as antibodies, serum antitrypsin proteins or serum proteolytic enzymes. Previous work in the laboratory has demonstrated the feasibility of this technique for quantitating the amount of enzyme required to induce emphysema in the intact animal and should be of considerable utility in the program.

The significance of this program will lie in its determination of the role played by smoking and other airborne pollutants on the development

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of emphysema. By studying the effects of the above agents on both the serum and lung antiproteinases we hope to demonstrate which of the two is more important in conditions likely to cause emphysema. We will also better understand the role of proteinase-antiproteinase balance during induction of experimental emphysema. We hope to utilize the observations made in our model system in understanding the sequence of events occurring during the development of human pulmonary emphysema. In accomplishing this we believe that we sahll be better able to describe those individuals most prone to emphysema development and, eventually, suggest a method of treatment.

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C. REFERENCES

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10. SPACE AND FACILITIES AVAILABLE:

The facilities available in the Biochemistry Laboratory for use during these investigations include:

1. Zeiss phase microscope
2. Sorvall centrifuge
3. Hitachi-Perkin Elmer spectrophotometer
4. Mettler balance
5. Refrigerator and freezer
6. Glassware for all the basic techniques described

The facilities available in the Cardiopulmonary Laboratories for use during these investigations include:

1. Eight-channel FM magnetic tape recorder with voice input.
2. Six-channel FM magnetic tape recorder with voice input.
3. Filtering and differentiator circuit.
4. Differential and vascular pressure transducers.
5. Animal body plethysmograph (modified small body respirator).
6. Spirometers.
7. Gas chromatograph for CO and N_2 analysis.
8. Godart CO Analyzer.
9. Godart CO_2 Analyzer.
10. Beckman O_2 Analyzer.
11. Blood gas and pH electrodes system with water bath and tonometer.
12. Pressure cycled respirator (Bird Mark VII).

The Research Laboratories have the following general facilities available:

1. Hotpack walk-in incubator
2. Walk-in cold room
3. RCA electron microscope
4. Dark room facilities

Animal boarding facilities occupy an adjoining building. An animal surgical suite is located there, contains a completely equipped operating facility and is maintained by a full-time staff. Standardization of dogs is practiced and during a three-week period of observation, testing and treatment prior to experimentation, pre-existing medical problems are eliminated.

11. ADDITIONAL FACILITIES REQUIRED:

NONE

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12. BIOGRAPHICAL SKETCHES

A. PHILIP KIMBEL, M.D.
Head, Pulmonary Disease Section
Born: REDACTED

Role in Project: Co-Principal Investigator

Education, Training, Honors

Temple University (Major in Sciences)
Temple University School of Medicine - M.D.
Internship, Albert Einstein Medical Center
Residency, Internal Medicine, Albert Einstein Medical Center
USPHS Post-Doctoral Research Fellowship
Department of Physiology and Pharmacology
Graduate School of Medicine
University of Pennsylvania

REDACTED

Professional Experience

Head, Pulmonary Disease Section, Albert Einstein Medical Center, Philadelphia, Pennsylvania	1961-
Professor of Medicine, Temple University Health Sciences Center, School of Medicine, Philadelphia, Pennsylvania	1971-
Associate Member, Research Laboratories, Albert Einstein Medical Center, Philadelphia, Penna.	1968-
Associate Professor of Medicine, Temple University Health Sciences Center School of Medicine.	1967-71
Associate in Medicine, Temple University Health Sciences Center School of Medicine	1963-67
Instructor in Medicine, Temple University Health Sciences Center School of Medicine	1960-63
Research Associate, Fels Research Institute, Temple University School of Medicine and Institute for Cancer Research (with Dr. S. Weinhouse - Blood Glucose Metabolism). Part-time	1958-61
Research Associate, Department of Physiology, Graduate School of Medicine, University of Pennsylvania (part-time). Worked with Dr. A. B. Dubois and Dr. H. Linderholm in studies of Pulmonary Capillary Blood Flow Simultaneously with Cardiac Catheterization	1958-61

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R: REDACTED MATERIAL

B. GEORGE WEINBAUM, Ph.D.
Bioscientist, Pulmonary Disease Section
Born: REDACTED

Role in Project: Co-Principal Investigator

Education, Training, Honors

University of Pennsylvania, Philadelphia, Pa. - A.B.
The Penna. State University, University Park, Penna. - M.S.
The Penna. State University, University Park, Penna. - Ph.D.
Tokyo U. Inst. Appl. Micro, Japan - Post-doctoral
Albert Einstein Medical Center, Philadelphia, Pa. Post-doctoral

Fulbright Research Scholar, Tokyo University
Career Development Awardee, Nat'l Inst. of Gen. Med. Sciences

REDACTED

Professional Experience

I am presently an Associate Member in the Biochemistry Department. My research at Albert Einstein Medical Center has involved studies on animal and bacterial cell membrane structure and synthesis, enzyme biosynthesis and regulation, biosynthesis of naturally occurring nucleoside analogs in fungi, abnormal cell wall synthesis and characterization of the lipids of E. coli cell wall complexes. I am a Career Development Awardee.

From 1957-61, I was director of the Biochemical Section of the Pathology Department at Geisinger Medical Center, Danville, Penna. My research involved amino acid analogs and tissue culture cells. I spent one year (1959-60) as a Fulbright Research Scholar at the Institute of Applied Microbiology in Tokyo University. I was studying exoenzyme synthesis in B. subtilis.

I received my Ph.D. from Penna. State Univ. in 1957, having worked with Dr. M. F. Mallette on induced enzyme synthesis in E. coli.

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R: REDACTED MATERIAL

C. BRUCE SLOAN, Ph.D.
Research Associate
Born: REDACTED

Role in Project: Research Associate

Education, Training, Honors

Temple University - B.A.
Hahnemann Medical College - M.S.
Hahnemann Medical College - Ph.D.
Department of Pathology, Harvard Medical School
Post-doctoral
Department of Microbiology, Albert Einstein Medical
Center - Post-doctoral

REDACTED

Temple University - Dean's list

Hahnemann Medical College, U.S.P.H.S. Predoctoral
Fellow

REDACTED

Professional Experience

I am presently a postdoctoral fellow in the Biochemistry Department, Pulmonary Disease Section, at Albert Einstein Medical Center. My research here has consisted of studies on proteolytic enzymes derived from dog polymorphonuclear leukocytes and macrophages. I have been attempting to assay, isolate and define the role of enzymes on the development of experimental emphysema using the dog as a model system.

From 1969-1971, I was a postdoctoral fellow in the Laboratory of Chemical Pathology, Department of Pathology, Harvard Medical School. My research was concerned with the role of genetics and the state of the antigen on cellular and humoral immune mechanisms.

I received my graduate training at Hahnemann Medical College, under Dr. Peter Stelos. My research was concerned with studies of the structure of immunoglobulin G, specific antibodies, and Bence-Jones proteins and employed enzymatic and chemical procedures for protein degradation and sequencing.

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13. PUBLICATIONS:

A. P. Kimbel, M.D.

Kaplan, A.S. and Kimbel, P.: Pulmonary Capillary Blood Flow Waves in Subjects with Abnormal Pulmonary Hemodynamics, Journal of Applied Physiology, 28:793 (1970).

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Source: https://www.industrydocuments.ucsf.edu/docs/jqyl0000

R: REDACTED MATERIAL

14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
George Weinbaum, Ph.D., Co-Principal Investigator	20	- 0 -
Philip Kimbel, M.D., Co-Principal Investigator	20	- 0 -
Bruce Sloan, Ph.D., Research Associate	50	REDACTED
Technical		
Biochemistry Technician	100	8,000
Histology Technician	100	8,500
Fringe Benefits		4,580
	Sub-Total for A	$28,080
B. Consumable supplies (by major categories)		
Dogs (approx. $50 each)		3,000
Boarding of animals ($1.25/day)		1,500
Rabbits (approx. $6 each)		300
Glassware		1,000
Chemicals, Drugs, Stains, etc.		2,000
	Sub-Total for B	$ 7,800
C. Other expenses (itemize)		
Travel to National Meetings		1,000
Books, Journals		300
Publication costs		500
	Sub-Total for C	$1,800
	Running Total of A + B + C	$37,680
D. Permanent equipment (itemize)		
2 Isolation chambers for pollutant exposure at $1,000 each		2,000
	Sub-Total for D	$ 2,000
E. Indirect costs (15% of A+B+C)	E	5,652
	Total request	$45,332

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	REDACTED	7,800	1,800	1,000	5,955	$46,655
Year 3		7,800	1,800	- 0 -	6,300	$48,300

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Source: https://www.industrydocuments.ucsf.edu/docs/jqyl0000

16. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Etiology of Experimental Emphysema	N.I.H. - HE 13787-01	$140,000	5/1/71 to 4/30/74
Phospholipid Metabolism in Membrane Synthesis	N.I.H. - NS-07268-06	$ 45,000	1/1/71 to 12/31/73
Structure, Function and Synthesis of Cell Membranes	N.I.H. - K04-GM-07259-04	$17,500	7/1/69 to 6/30/74

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Proteinase:Antiproteinase Balance in the Lung and the Effect of Air-borne Pollutants	National Tuberculosis Association (applied for 11/72)	$22,678	5/1/73 to 4/30/74

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal Investigator 3:

Typed Name George Weinbaum, Ph.D.

Signature ~~Philip Kimbel, M.D.~~ Date 1/15/73

Telephone 215 (Area Code) DA 9 0700 (Number) 363 or 463 (Extension)

Checks payable to

Jerome Baron, Vice-President for Fiscal Affairs

Mailing address for checks

Albert Einstein Medical Center - N.D.
York & Tabor Roads, Phila., Pa. 19141

Responsible officer of institution

Typed Name Mr. Bertram Zimmerman

Title Acting General Director

Signature [signature] Date 1/25/73

Telephone 215 (Area Code) DA 9-0700 (Number) 381 (Extension)

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